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Research Article

STANDARDIZATION AND CONTAMINATION STUDIES ON NUTGALLS OF *QUERCUS INFECTORIA* OLIVIER

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ABSTRACT

Drug consists of nutgalls of *Quercus infectoria* (Family-Fagaceae), commonly known as "Mazuphal". Standardization parameters like, physical constants, ash content, solvent residues, phytochemical screening, fluorescence and microscopical analysis were carried out for the quality, strength and purity of the drug. The present work is an also attempt to evaluate contamination parameters for safety of an herbal drug *Quercus infectoria* such as microbial contamination, aflatoxins, pesticide residues and heavy metals. Result revealed that the total ash content and acid insoluble siliceous matter were found 2.11% and 0.22% respectively in the drug. The extractive values were varies at different solvent at different condition. Loss on drying and pH at 25(ºC) were 9.97 and 6.1 respectively. Study revealed that total bacterial count and total fungal count were found to be within the permissible limit. Heavy metal like cadmium and mercury were not detected in the drug but arsenic (0.31 mg/kg) and lead (0.70 mg/kg) were found under permissible limit. Pesticide residue such as o, p- DDD, p, p- DDD, o p- DDE, p, p- DDE, o, p- DDT, p, p- DDT, α- Endosulfan, β – Endosulfan, Parathion, α- HCH, β- HCH, γ- HCH, δ- HCH was absent in the nutgalls.

Keyword: *Quercus infectoria*, Standardization, Contamination

INTRODUCTION

Quercus infectoria Olivier (Fagaceae) is a small tree or shrub mainly found in Greece, Asia Minor, Syria, and Iran. The tree bears galls that emerge on its shoots as a consequence of assault of gall wasp. *Cypnis gallae-tincotoriae.* Pharmacological evaluation of the galls of *Q. infectoria* has indicated them to be astringent, antidiabetic, antitremorine, local anesthetic, anticandidicidal[1, 2] anti-inflammatory[3] antibacterial and antifungal[4]. In Asian countries, the galls of *Q. infectoria* have been used for centuries in oriental traditional medicines for treating inflammatory diseases[5, 6, 7]. The phytochemical studies carried out so far have revealed the presence of tannic acid (gallotannic acid, the principal constituent. 50-70%). gallic acid, syringic acid, ellagic acid, f-sitosterol, amentoflavonc hexamcthyl ether, isocryptomerin, starch, essential oils, anthocyanins, methyl-betulate, methyl-oleanate, hexagalloylglucose[2], and polygalloyl-glucose[8, 9] in *Q. infectoria* galls. The present study deal with standardization of the nutgalls of *Quercus infectoria* Olivier. (Fagaceae), by using microscopical, physical and contamination profile parameters as per WHO guidelines.

MATERIAL AND METHODS

Collection and authentication of crude drug

The sample was purchases from a Unani drug supplier with the knowledge of Unani physician and authenticated by Dr. H.B. Singh, Scientist F, Head of Raw Material Herbarium and Museum (RHMD), National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India (Ref. No. NISCAIR/RHMD/Consult/2011-12/1822/122).

Organoleptic properties

Proper examination of the untreated sample of Nutgall of the chosen plant was carried out under diffused sunlight and artificial source similar to day light.

Powder microscopy

Powdered nutgalls was taken and it was cleared with chloral hydrate solution and stained with Phlouroglucinol and conc. HCl. Powder is mounted with glycerine and observe under microscope[10].

Physical parameters

Extractive value and ash value of nutgalls was carried out by following standard WHO techniques. Fluorescence analysis was carried out by Chase and Pratt methods[11]. Qualitative phytochemical tests were carried out by standard methods[12]. pH and loss on drying determination of nutgalls was carried out according to Indian Pharmacopoeia[13].

Contamination study

Microbial load, pesticidal residue, heavy metal analysis and aflatoxin determination was carried out according to Pharmacopoeial standards[14-16].

RESULTS AND DISCUSSION

Organoleptic properties

The organoleptic properties of nutgalls are globular in shape, 0.8 cm to 2.5 cm in diameter, green-yellow in colour; odour is slight, strongly pungent taste and tuberculated surface. All these parameters are more important for morphological identification.

Powder microscopy

Yellow-brown powder showed parenchyma made up of rounded to polygonal cells with thickened and pitted walls and distinct intercellular spaces. Sclereids vary in shape but are usually more or less ovoid to rectangular. Calcium oxalate crystal found scattered as well as in parenchymatous tissue. Starch granules found massed together in groups, individual granule fairly large ovoid to spherical or indistinctly polyhedral with a well marked radiate or slit shaped hilum. Lignin vessels found in groups of small, with spiral or annular thickening. Lignin bodies scattered as brown globular masses which gives positive reaction for lignin (Fig 2).

Physical parameters

All the phytochemical standards were established according to procedure laid down in WHO guidelines and Indian Pharmacopoeia. The total and acid insoluble ash values of powdered drug were found 2.11% and 0.22% respectively which under the limit of UPI and API (Table 1). The analysis of ash suggested the presence of inorganic substances in considerable normal amount which was indicated that adulterated materials (silica, sand, dust and soil) were not present in the drug. The pH of 1% the formulation was found to be 6.1 (Table 1). The drug was slightly acidic in nature because of the pH value of 1% solution was 6.1. The alcoholic extractive value (64.9182%) was more as compared to other solvents like petroleum ether, chloroform, acetone

etc. that means the chemical constituent of the drug more soluble in alcoholic solvent. The hot extractive value was more as compared to successive and cold in all solvent (Table 2).

Loss on drying value of formulation was found 9.97 and if it is in more amounts then drug is more prone for microbial infections (Table 1).

Fluorescence analysis was carried out to check purity of the drug. The powder form of nutgalls was observed in visible light light brown in colour. The powder was treated with different type of reagents and observed in UV light (short and long wavelenght) and it emit different colour fluorescence as shown in (Table 3).

Qualitative analysis of drug indicated the presence of Alkaloids, Glycosides, Tannin, Resins, Lipids/fats, Phenolics and Flavonoids coumpounds (Table 4).

Contamination evaluation

Microbial load is one of important parameter which mentioned in WHO to determine the quality of the formulation for medication. Microbial load of the preparation was found negative for the presence of bacterial and fungal indicate the good quality of the product. Heavy metals such as lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) are natural constituents of the environment like air, water and soil.

Safety profile of drug was found out. The drug showed the results within prescribed limits (as per WHO guideline). Therefore the drug was found to be free of lethal effect due to absence or under the limits of these safety parameters such as microbial load determination (Table 5.figure 3) Heavy metal analysis (Table 6), Aflatoxin residue (Table 7) and Pesticidal residue (Table 8) were indicating that nutgall was safe to administer.

CONCLUSION

Powder microscopy of drug showed the presence of parenchyma, stone cell, parenchyma with starch and tannin bodies, lignin bodies, starch granules etc. in the powder of nutgalls of *Quercus infectoria* which are also mentioned in UPI and API. The physic-chemical parameters like ash values, extractive values, flourescence analysis, pH determination, loss on drying, qualitative estimation of phyto-constituents of this drug was established and safety profile parameters (microbial load determination, heavy metal analysis, aflatoxin residue and pesticidal residue) of standardization were also determined and was found to be under the limit (as per WHO guidelines) which indicate that nutgalls of *Quercus infectoria* was safe. The present study will be useful for the establishment of quality standards and may be useful for the further research on nutgalls of *Quercus infectoria* Olivier. for academicians and researchers.

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REFERENCES

1. Dar, M.S., Ikram, M., Fakouhi, T., 1976. Pharmacology of *Quercus infectoria*. J Pharm Sci. 65, 1791-1794.
2. Hwang, J.K., Kong, T.W., Baek, N.I., Pyun, Y.R., 2000. α- Glycosidase Inhibitory Activity of hexagalloylglucose from the galls of *Quercus infectoria*. Planta Med. 66, 273-274.
3. Kaur, G., Hamid, H., Ali, A., Alam, M.S., Athar, M., 2004. Antiinflammatory evaluation of alcoholic extract of galls of *Quercus infectoria*. J. Ethnopharmacol. 90, 285-292.
4. Basri, D.F., Tan, L.S., Shafiei, Z., Zin, N.M., 2012. In Vitro Antibacterial Activity of Galls of *Quercus infectoria* Olivier against Oral Pathogens. Evidence-Based Complementary and Alternative Medicine. 3, 423-439.
5. Anonymous. 1995. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products, 13 volume series. Publications & Information Directorate (CSIR), New Delhi. 351.
6. Chopra, R.N., Nayar, S.I., Chopra, I.C., 1956. Glossary of Indian Medicinal Plant. Council of Scientific and Industrial Research, India. 208.
7. Galla, B.P., 1911. Galls. The British Pharmaceutical Codex. Council of the Pharmaceutical Society of Great Britain.
8. Ikram, M., Nowshad, F., 1977. Constituents of *Quercus infectoria*. Planta Med. 31, 286-287.
9. Dar, M.S., Ikram, M., 1979. Studies on *Quercus infectoria*; isolation of syringic acid and determination of its central depressive activity. Planta Med. 35, 156–161.
10. Anonymous. 2002. Quality control method for medicinal plants materials, WHO, Geneva, AITBS publisher and distributers, Delhi. 28-73.
11. Chase, J.A., Pratt, R.J., 1949. Flourescence of powder vegetables drugs with particular reference to development of system of identification. J. Ame Pharmaceut Associ. 38, 324-331.
12. Fransworth, N.R., 1996. Biological and phytochemical screening of plants. J.Pharm.Sci. 55, 225-272.
13. Anonymous. 1996. Indian Pharmacopoeia, Ministery Of Health and Family Welfare, Controller of publication, Govt. of India, New Delhi. Vol-2, A-53, A-54.
14. Anonymous. 1996. Indian Pharmacopoeia, Ministery Of Health and Family Welfare, Controller of publication, Govt. of India, New Delhi. A-110, A-111, A-115.
15. Haque, M.R., Ansari, S.H., Naquvi, K.J., Najmi, K.A., 2012. Quality assessment of a traditional unani formulation. JPR. 5(2), 778-782.
16. Anonymous. 2002. Association of Official Analytical Chemists (AOAC), 17th edition methods AOAC 970.33, methods AOAC 990.33.

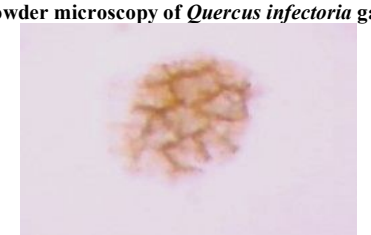
Morphology of Nutgalls of *Quercus infectoria*

Fig- 1: Morphology of Nutgalls

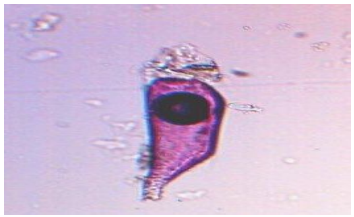
Organoleptic properties

Shape	Globular
External colour	Green-yellow
Size	0.8-2.5cm
Surface	Tuberculated
Texture	no hair
Odour	slight
Taste	strongly astringent
Thickness	0.2mm

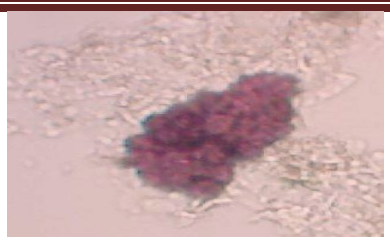
Powder microscopy of *Quercus infectoria* galls

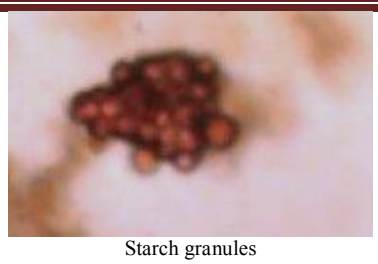


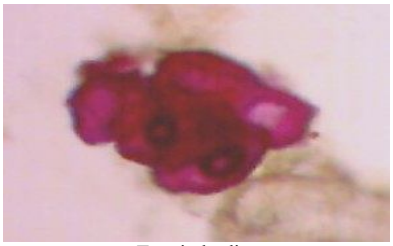
Parenchyma



Stone cell


Parenchyma with starch and tannin bodies


Starch granules


Tannin bodies


Lignin bodies

Fig 2. Powder microscopy of *Quercus infectoria* galls.

Table 1: Ash value, pH and Loss on drying of *Quercus infectoria* galls

Parameters	Mean
Ash Value	
Total ash	2.11**(% w/w)**
Acid insoluble ash	0.8 **(% w/w)**
pH of 1% solution at pH at 25 (° C)	6.1
Loss on drying	9.97**(% w/w)**

Table 2. Extractive value of *Quercus infectoria* galls.

Extract	Pet.ether	Chloroform	Acetone	Alcohol	Hydro-Alcoholic	Aqueous
Hot %	2.6	2.989	3.776	64.9182	45.386	51.3211
Cold %	1.63	2.65	7.33	20.068	16.40	19.01
Successive%	0.12	3.63	3.69	20.22	10.23	12.877

Table 3. Flourescence analysis of *Quercus infectoria* galls.

S.No	Treatment	Day Light	UV Light (254)	UV Light (366)
1	Powder as such	Light brown	brown	Light brown
2	Powder treated with dist. water	Light brown	brown	Yellowish-brown
3	Powder treated with 5% NaOH	Reddish-brown	black	black
4	Powder treated with H_2SO_4	Light brown	Dark brown	brown
5	Powder treated with conc. HCl	Brown	black	Yellowish-brown
6	Powder treated with acetone	Light green	Dark red	Light green
7	Powder treated with chloroform	Greenish yellow	Red	Dark green
8	Powder treated with conc. HNO_3	Orange	black	Yellowish-brown
9	Powder treated with $FeCl_3$	Bluish-black	black	Bluish-black
10	Powder treated with pet ether	Light green	Red	Light green
11	With ammonia solution	Reddish- brown	black	black
12	With picric acid	Yellowish-brown	black	Greenish-yellow

Table 4: Qualitative test of *Quercus infectoria* galls.

Extract Constituents	Pet ether	Acetone	Chloroform	Alcoholic	Hydro-alcoholic	Aqueous
Alkaloids	-	-	-	+	++	+++
Carbohydrates	-	-	-	-	-	-
Glycosides	-	+	+	-	++	+
Tannin	+	+	+	+	+	+
Phenolic	+	+	+	++	++	++
Flavonoids	-	+	++	+	++	+
Proteins & A.A	-	-	-	-	-	+
Resins	-	++	+	++	-	-
Lpids/Fats	+	+	-	+	-	-

(-Absent, +Present, ++highly present)

Table 5. Microbial load determination of the *Quercus infectoria* galls

S. No.	Parameter Analyzed	Results	WHO Limits
1	Total Bacterial Count	54 CFU/gm	10^5 CFU /gm
2	Total Fungal Count	<10	10^3 CFU /gm

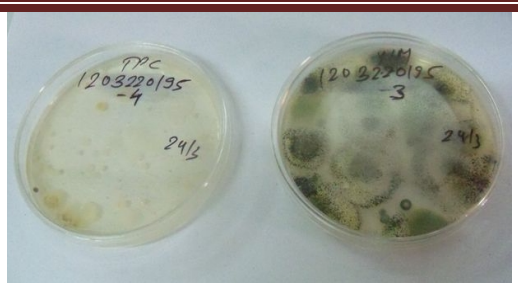


Fig- 3: Microbial load determination (TPC/TBC and TFC)

Table 6. Heavy Metal of *Quercus infectoria* galls (Ph. Eur. Chapter 2.2.58.)

S. No	Test Parameter	Test Method	Result
1.	Cadmium (Cd)	ICP-OES	Not Detected
2.	Lead (Pb)	ICP-OES	0.70 mg/kg
3.	Arsenic(As)	ICP-OES	0.31 mg/kg
4.	Mercury(Hg)	ICP-OES	Not Detected

Table 7. Aflatoxin determination of *Quercus infectoria* galls by AOAC 990.33 method.

S. No.	Test Parameter	Test Method	Result
1.	Aflatoxin B1	AOAC 990.33	Not Detected
2.	Aflatoxin B2	AOAC 990.33	Not Detected
3.	Aflatoxin G1	AOAC 990.33	Not Detected
4.	Aflatoxin G2	AOAC 990.33	Not Detected

Table 8. Pesticidal residue of *Quercus infectoria* galls by AOAC 970.33 method.

S. No	Pesticides	Test Method	Result
1.	α-BHC	AOAC 970.52/EPA 525.2	Not Detected
2	β-BHC	AOAC 970.52/EPA 525.2	Not Detected
3	γ-BHC(Lindane)	AOAC 970.52/EPA 525.2	Not Detected
4	δ-BHC	AOAC 970.52/EPA 525.2	Not Detected
5	Heptachlor	AOAC 970.52/EPA 525.2	Not Detected
6	Heptachlor_Epoxide	AOAC 970.52/EPA 525.2	Not Detected
7	α-Chlordane	AOAC 970.52/EPA 525.2	Not Detected
8	α-Endoulfan	AOAC 970.52/EPA 525.2	Not Detected
9	β-Chlordance	AOAC 970.52/EPA 525.2	Not Detected
10	Endrin	AOAC 970.52/EPA 525.2	Not Detected
11	Total DDE	AOAC 970.52/EPA 525.2	Not Detected
12	Total DDD	AOAC 970.52/EPA 525.2	Not Detected
13	Total DDT	AOAC 970.52/EPA 525.2	Not Detected
14	β-Endoulfan	AOAC 970.52/EPA 525.2	Not Detected
15	Parathion	AOAC 970.52/EPA 525.2	Not Detected
16	2,4-D	PAM Vol I / EPA 515.3	Not Detected

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